

What is claimed is:

1. A method of assaying a sample chosen from heparin, low-molecular-weight heparin, ultra low molecular weight heparin, and oligosaccharides, wherein the sample is analysed using a reversed phase column coated with a quaternary ammonium salt for chromatographic separation and analysis of a complex mixture of oligosaccharides.
2. The method according to claim 1, wherein the reversed phase column is a C₈ or C₁₈ column.
3. The method according to claim 1, wherein all of the oligosaccharides from disaccharides to dodecasaccharides are detected.
4. The method according to claim 2, wherein the sample is analysed without pre-treatment using the C₈ or C₁₈ reversed phase column coated with a quaternary ammonium salt.
5. The method according to the claim 2, wherein the C₈ or C₁₈ reversed phase column coated with a quaternary ammonium salt is a cetyl trimethylammonium-strong anion exchange (CTA-SAX) column.
6. The method according to claim 2, wherein the C₈ or C₁₈ reversed phase column coated with a quaternary ammonium salt is a CTA-SAX column.
7. The method according to claim 2, wherein the sample is fractionated by Gel Permeation Chromatography (GPC) prior to the chromatographic separation using the C₈ or C₁₈ reversed phase column coated with a quaternary ammonium salt.
8. The method according to claim 7, wherein the C₈ or C₁₈ reversed phase column coated with a quaternary ammonium salt is a CTA-SAX column.

9. The method according to claim 2, wherein the sample is depolymerized either partially or totally prior to the chromatographic separation using the C₈ or C₁₈ reversed phase column coated with a quaternary ammonium salt.

10. The method according to claim 2, wherein the sample is reduced after it is depolymerised and prior to the chromatographic separation using the C₈ or C₁₈ reversed phase column coated with a quaternary ammonium salt.

11. The method according to the claim 9, wherein the C₈ or C₁₈ reversed phase column coated with a quaternary ammonium salt is a CTA-SAX column.

12. The method according to the claim 10, wherein the C₈ or C₁₈ reversed phase column coated with a quaternary ammonium salt is a CTA-SAX column.

13. A method of assaying a sample chosen from heparins, low-molecular-weight heparins, ultra low molecular weight heparins, and oligosaccharides comprising:

- (a) depolymerizing the sample by enzymatic depolymerization
- (b) reducing the depolymerized sample; and
- (c) assaying the sample of step (a) and/or step (b) by CTA-SAX

chromatography.

14. The method according to claim 13, wherein the sample is enzymatically depolymerised using at least one heparinase.

15. The method according to claim 14, wherein the at least one heparinase is chosen from heparinase 1 (EC 4.2.2.7.), heparinase 2 (heparin lyase II), and heparinase 3 (EC 4.2.2.8.).

16. The method according to claim 14, wherein the sample is enzymatically depolymerised using a mixture of heparinases comprising heparinase 1 (EC 4.2.2.7.), heparinase 2 (heparin lyase II), and heparinase 3 (EC 4.2.2.8.).

17. The method according to claim 10, wherein the sample is reduced by NaBH_4 or by an alkali metal salt of the borohydride anion

18. The method according to claim 1, wherein the low-molecular weight heparin is enoxaparin sodium.

19. The method according to claim 10, wherein the sample is enoxaparin sodium and said sample is reduced to reduce the reducing ends of said enoxaparin sodium which are not in the 1,6-anhydro form.

20. The method according to claim 1, wherein the chromatographic separation uses a mobile phase which is transparent to UV light having wavelengths in the range of about 200 nm to about 400 nm.

21. The method according to claim 1, wherein the chromatographic separation uses a mobile phase comprising methane sulfonate salts.

22. The method according to claim 1, wherein the chromatographic separation uses a mobile phase comprising ammonium methane sulfonate or sodium methane sulfonate.

23. The method according to claim 2, wherein chromatography on the C_8 or C_{18} reversed phase column coated with a quaternary ammonium salt is performed at a pH of about 2.0 to about 7.0

24. The method according to claim 2, wherein chromatography on the C₈ or C₁₈ reversed phase column coated with a quaternary ammonium salt is performed at a pH of about 2.5 to about 3.0.

25. The method according to claim 2, wherein chromatography on the C₈ or C₁₈ reversed phase column coated with a quaternary ammonium salt is performed in a mobile phase comprising water adjusted to about pH 3 by adding methane sulfonic acid and/or 2 M ammonium methane sulfonate, at about pH 2.5.

26. The method according to claim 1, further comprising a step for detecting the presence of oligosaccharide chains whose end is modified with a 1,6-anhydro bond.

27. The method according to claim 1, further comprising a step for detecting acetylated sugars.

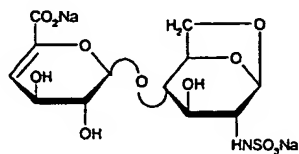
28. The method according to claim 27, wherein acetylated sugars are detected by subtracting an absorbance measured at a wavelength at which both acetylated and nonacetylated sugars absorb from an absorbance measured at a wavelength at which acetylated but not nonacetylated sugar absorbs.

29. The method according to claim 28, wherein acetylated sugars are selectively detected.

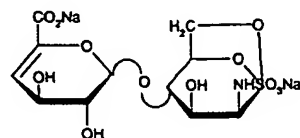
30. The method according to claim 28, wherein the acetylated sugars detected are at least one acetylated oligosaccharide chosen from Δ IVa, Δ IIa, Δ IIIa, Δ Ia, Δ IIa-IVs_{glu}, and Δ IIa-IIIs_{glu}

31. The method according to claim 1, wherein the low-molecular weight heparin is any LMWH seeking approval by a regulatory authority pursuant to an application citing Lovenox[®]/Clexane[®] (enoxaparin sodium injection) as the listed drug.

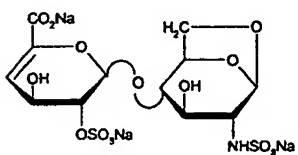
32. The method of claim 1 wherein said analysis leads to detection of at least one saccharide chosen from any of the following four saccharides



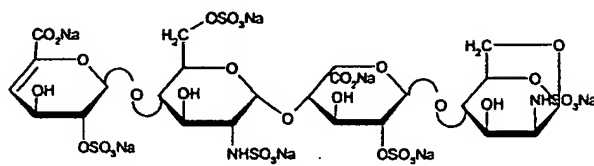
disaccharide 1



disaccharide 2

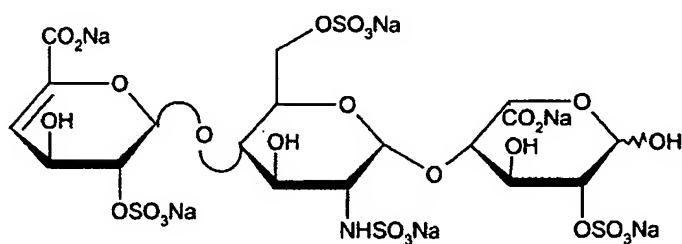


disaccharide 3



tetrasaccharide 1

33. The method of claim 1 wherein said analysis leads to detection of



trisaccharide 1

34. The method of claim 1 wherein said analysis leads to detection of at least one saccharide chosen from any of the following saccharides:

